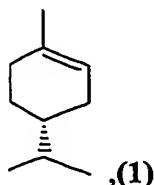


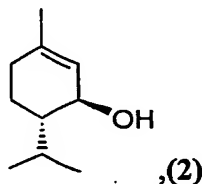
WHAT IS CLAIMED IS:

1. A method for making (-)-menthol, the method comprising:

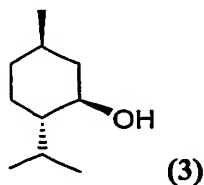
a) providing starting material comprising 4R(+)-1-menthene having formula (1):



b) oxidizing the starting material in the presence of a catalyst comprising at least one polypeptide capable of hydroxylating at least one enantiomer of 1-menthene or limonene at an allylic carbon C(3), thereby forming a hydroxylated menthene product comprising at least 50% of the *trans*-piperitol having formula (2):



c) hydrogenating the *trans*-piperitol of formula (2) in the presence of a catalyst to form (-)-menthol having at least about 62% chemical purity, and at least about 90% enantiomeric excess, said (-)-menthol represented by formula (3):



2. The method of claim 1, wherein 4R(+)- menthene in the starting material has optical purity of at least about 90%.

3. The method of claim 1, wherein 4R(+)-1-menthene in the starting material is derived from partial hydrogenation of orange or other citrus oil comprising at least about 90% of 4R(+)-limonene.

4. The method of claim 1, wherein the starting material comprises a mixture of 4R(+)-1-menthene and 4R(+)-limonene in the ratio between about 1:100 to about 100:1.

5. The method of claim 1, wherein the polypeptide has limonene-3-hydroxylase activity.

6. The method of claim 1, wherein the polypeptide is encoded by nucleic acids isolated from at least one species of plant of genera selected from *Lamiaceae*, *Umbelliferae*, *Asteraceae*, *Rutaceae*, *Rosaceae*, *Myrtaceae*, *Gramineae*, *Geranaceae*.

7. The method of claim 1, wherein the polypeptide is encoded by nucleic acids isolated from at least one species of fungi or bacteria.

8. The method of claim 7, wherein species of fungi or bacteria are selected from *Hormonema*, *Aspergillus*, *Pseudomonas*, *Rhodococcus*, *Bacillus*.

9. The method of claim 1, wherein the oxidation step b) is carried out by at least one polypeptide encoded by modified nucleic acids, and wherein modifications of nucleic acids have been obtained by:

a) at least one method selected from mutagenesis, recombination, gene shuffling, combinatorial gene synthesis, and

b) at least one screening method allowing for detection of *trans*-piperitol (2) or *trans*-isopiperitenol (5) in the presence of other oxygenated menthene or limonene derivatives.

10. The method of claim 1, wherein the oxidation step b) is carried out by at least one polypeptide encoded by nucleic acids isolated from bacteria or fungi capable of

growth on at least one compound selected from camphor, borneol, isoborneol, 1,8-cineole, 1,4-cineole, fenchone, fenchol, terpineol, terpin hydrate and mixtures thereof.

11. The method of claim 1, wherein the oxidation step b) is carried out using hemoprotein enzyme as catalyst and hydrogen peroxide.

12. The method of claim 1, wherein the oxidation step b) is carried out in the presence of water-immiscible liquid organic solvent comprising 4R(+)-1-menthene, wherein said organic solvent to water ratio is at least about 1:50 by volume.

13. The method of claim 1, wherein the biological oxidation step b) is carried out by a polypeptide having regioselectivity for 3-*trans*-hydroxylation of R(+)-1-menthene (1) or 4R(+)-limonene (4) of at least about 75%.

14. The method of claim 1, wherein the biological oxidation step b) is carried out by a polypeptide having regioselectivity for 3-*trans*-hydroxylation of R(+)-1-menthene (1) or 4R(+)-limonene (4) of at least about 90%.

15. The method of claim 1, wherein the hydrogenation step c) is carried out using a catalyst selected from the group comprising platinum, palladium, palladium oxide, combinations thereof, and salts thereof.

16. The method of claim 15 wherein the catalyst comprises a substantially insoluble inorganic support selected from at least one salt of alkali earth metal and at least one acid, said acid being selected from the group comprising carbonic, phosphoric, and sulfuric and mixtures thereof, or substantially insoluble support selected from silica, alumina, titania, zirconia, carbon, or mixtures thereof.

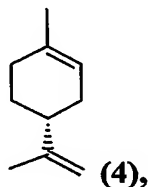
17. The method of claim 1, wherein the hydrogenation step c) is carried out using a catalyst comprising at least one of palladium or palladium oxide, salts thereof, and calcium carbonate.

18. The method of claim 1, wherein the hydrogenation step c) is carried out in the absence of solvent or in the presence of at least one solvent selected from the group comprising water, alcohol or glycol or polyol, said alcohol or glycol or polyol having linear or branched or cyclic alkyl or alkyloxyalkyl chain having from 1 to 20 carbon atoms.

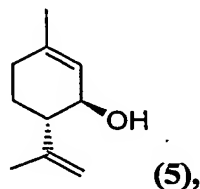
19. The method of claim 1, wherein biological oxidation step b) is carried out using a monooxygenase enzyme in an electrochemical reactor.

20. A method for making (-)-menthol, the method comprising:

a) providing starting material comprising 4R(+)-limonene having formula (4):

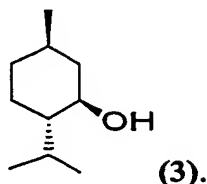


b) oxidizing the starting material in a presence of catalyst comprising at least one polypeptide capable of *trans*-hydroxylation of at least one enantiomer of 1-menthene or limonene at an allylic carbon C(3), thereby forming hydroxylated menthene product comprising at least about 75% of the *trans*-isopiperitenol having formula (5)



c) hydrogenating the *trans*-isopiperitenol of formula (5) in the presence of a catalyst, wherein hydrogenation selectivity of the said catalyst provides for (-)-menthol formation with a ratio of (-)-menthol to (-)-isomenthol of at least about 70:30, thereby forming (-)-menthol having at least about 70% chemical purity,

and at least about 90% enantiomeric excess, said (-)-menthol represented by formula (3):



21. The method of claim 20, wherein 4R(+)-limonene in the starting material has optical purity of at least about 90%.

22. The method of claim 20, wherein the starting material comprises essential oil of orange or other citrus fruit, said oil containing at least about 90% of 4R(+)-limonene.

23. The method of claim 20, wherein the starting material comprises a mixture of 4R(+)-1-menthene and 4R(+)-limonene in the ratio between about 1:100 to about 100:1.

24. The method of claim 20, wherein the polypeptide has R(+)-1-menthene 3-monooxygenase activity.

25. The method of claim 20, wherein the polypeptide is encoded by nucleic acids isolated from at least one species of plant of genera selected from *Lamiaceae*, *Umbelliferae*, *Asteraceae*, *Rutaceae*, *Rosaceae*, *Myrtaceae*, *Gramineae*, *Geranaceae*.

26. The method of claim 20, wherein the polypeptide is encoded by nucleic acids isolated from at least one species of fungi or bacteria.

27. The method of claim 26, wherein species of fungi or bacteria are selected from *Hormonema*, *Aspergillus*, *Pseudomonas*, *Rhodococcus*, *Bacillus*.

28. The method of claim 20, wherein the oxidation step b) is carried out by at least one polypeptide encoded by modified nucleic acids wherein modifications of nucleic acids have been obtained by using:

- a) at least one method selected from mutagenesis, recombination, gene shuffling, combinatorial gene synthesis, and
- b) at least one screening method allowing for detection of *trans*-piperitol (2) or *trans*-isopiperitenol (5) in the presence of other oxygenated menthene or limonene derivatives.

29. The method of claim 20, wherein the oxidation step b) is carried out by at least one monooxygenase encoded by nucleic acids isolated from bacteria or fungi capable of growth on at least one compound selected from camphor, borneol, isoborneol, 1,8-cineole, 1,4-cineole, fenchone, fenchol, terpineol, terpin hydrate and mixtures thereof.

30. The method of claim 20, wherein the oxidation step b) is carried out using hemoprotein enzyme as catalyst and hydrogen peroxide.

31. The method of claim 20, wherein the oxidation step b) is carried out in the presence of water-immiscible liquid organic solvent comprising 4R(+)-limonene wherein said organic solvent to water ratio is at least about 1:50 by volume.

32. The method of claim 20, wherein the biological oxidation step b) is carried out by monooxygenase having regioselectivity for 3-*trans*-hydroxylation of 4R(+)-limonene (4) or R(+)-1-menthene (1) of at least about 75%.

33. The method of claim 20, wherein the biological oxidation step b) is carried out by monooxygenase having regioselectivity for 3-*trans*-hydroxylation of 4R(+)-limonene (4) or of R(+)-1-menthene (1) of at least about 90%.

34. The method of claim 20, wherein the hydrogenation step c) is carried out using hydrogenation catalyst selected from the group consisting of palladium, palladium oxide, combinations thereof and salts thereof.

35. The method of claim 34, wherein the catalyst comprises a substantially insoluble inorganic support selected from at least one salt of an alkali earth metal and at least one acid, said acid being selected from the group comprising carbonic, phosphoric, or sulfuric, and mixtures thereof, or substantially insoluble support selected from silica, alumina, titania, zirconia, carbon, or mixtures thereof.

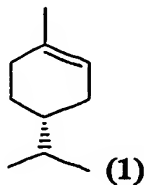
36. The method of claim 20, wherein the hydrogenation step c) is carried out using a catalyst comprising at least one of palladium and palladium oxide, salts thereof, and calcium carbonate.

37. The method of claim 20, wherein the hydrogenation step c) is carried out in the absence of solvent or in the presence of at least one solvent selected from the group consisting of water, of alcohol or glycol or polyol, said alcohol or glycol or polyol having linear or branched or cyclic alkyl or alkyloxyalkyl chain having from 1 to 20 carbon atoms.

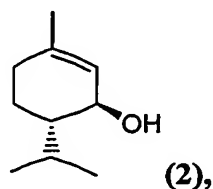
38. The method of claim 1, wherein the oxidation step b) is carried out using a monooxygenase enzyme in an electrochemical reactor.

39. A method for making (-)-menthol, comprising:

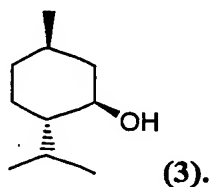
a) biologically oxidizing a starting material comprising 4R(+)-1-menthene having formula (1):



in the presence of at least one polypeptide to form a product comprising at least 50% of the *trans*-piperitol having formula (2):



b) hydrogenating the *trans*-piperitol of formula (2) in the presence of a catalyst to form the (-)-menthol of formula (3), wherein the (-) menthol has a chemical purity of at least about 62%, and wherein the (-)-menthol is formed in at least about 90% enantiomeric excess:



40. The method of claim 39, wherein the (-)-menthol has a chemical purity of at least about 70%.

41. The method of claim 39, wherein the (-)-menthol has a chemical purity of at least about 72%.

42. The method of claim 39, wherein the catalyst is selected from the group consisting of platinum, palladium, palladium oxide, combinations thereof and salts thereof.

43. The method of claim 42, wherein the catalyst is selected from at least one of palladium and palladium oxide immobilized on calcium carbonate.